# A NEW WATER SOLUBLE ACTIVE CONSTITUENT OF SQUILLS

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Notwithstanding the extensive therapeutic employment of squill and the pharmacological interest attaching to it on account of its relation to the digitalis series of heart poisons the work published on this subject from the chemical point of view is very scanty.

In 1879 Jarmersted (Archiv. f. exp. Path. u. Pharm., xi, 22) described an amorphous glucosidal toxic product "scillaïn" which was very little soluble in water but readily soluble in alcohol. In the same year E. Merck (Pharm. Zeit., 1879, no. 38) described three active constituents of squill, namely, scillitoxin, scillipicrin, and scillin. Of these scillitoxin may be considered to be practically identical with Jarmersted's "scillaïn," and a dose of from 0.12 to 0.13 mg. injected into a frog was sufficient to cause death. Neither of these products however can be considered to represent the active principle of squill in a pure form.[1] Scillipicrin is very feebly active (10 to 20 mg. being necessary to cause death in the frog) while scillin is even less active. Since these publications no communication of any importance has appeared, although Waliszewski in 1894 (L'Union Pharm., xxxiv, 251) claimed to have isolated three crystallisable active principles which he termed scillinine, scillipicrine, and scillimarine. His work however is unsupported by any experimental detail and no further communication from this source has appeared.

[1]A commercial specimen of scillitoxin was easily shown to be far from a pure product. In the first place the product is not wholly readily soluble in absolute alcohol and merely precipitating the alcoholic solution with ether yielded a product which was about twice as toxic as the original preparation.

During the last two years experiments have from time to time been carried out in these laboratories with the object of isolating in a crystalline form the active principle (or principles) of this drug and although the primary object of the investigation has so far not been achieved the results obtained appear to be of sufficient interest and importance to be worthy of record.

The most interesting result of the work has been to show that the activity of squill is due in part at least to a water-soluble glucoside which somewhat resembles the strophanthins from strophanthus hispidus and strophanthus kombe both from the chemical point of view, with regard to its solubilities, glucosidic nature, and colour reactions, and from the pharmacological side on account of its high degree of toxicity and similarity of action. The minimum lethal dose of this substance is 0.03 mg. for an average sized frog (25 grams) death occurring within three hours after injection, the heart being arrested in systole. This minimum lethal dose is thus about equal to that of either of the strophanthins mentioned above, somewhat less than that of digitoxin in its purest form, and almost half that of apocynamarin the recently isolated crystalline active principle of apocynum (Moore, Trans. Chem. Soc., 1909, 95, 735; Finnemore, Proc. Chem. Soc., 1909, 25, 77).

The most suitable method of preparation of this highly toxic substance is set out below. By the method employed the whole of the original activity of the squills tincture was obtained in the form of a solid the toxicity of which was about three times as great as that of Merck's scillitoxin.

Ten litres of concentrated Tinctura Scillae (5 × B.P.) were shaken out six times with, each time, half this volume of a mixture of chloroform (9 volumes) and alcohol (1 volume). The extracts were mixed, washed with small quantities of dilute sodium carbonate solution until the washings were alkaline, then with water until neutral, and finally evaporated to dryness under diminished pressure. There was thus obtained 21 grams of a dark brown solid which was found to represent practically the whole of the original activity of the tincture.

In order to determine the activity of the various fractions produced the following procedure was employed. The minimum lethal dose of each fraction was determined, as that amount of substance per 100 gram of body weight which would cause the death of a frog within three hours after injection into the dorsal lymph sacs, the heart being arrested in systole. These very numerous determinations have in all cases been carried out by Dr. P. P. Laidlaw of these laboratories, to whom the author wishes to express his indebtedness for this very necessary help, and for many valuable suggestions during the course of the investigation. It was thus found that the m. l. d. of the crude product obtained as described above was approximately 0.20 mgs. and, as already stated, represented the original activity of the tincture almost quantitatively.

The solid was dissolved in about 100 cc. of hot absolute alcohol in which it was almost completely soluble. On standing a very small quantity of a substance which proved to be inactive, and was not further examined, separated. The alcoholic solution was poured into about 1500 cc. of dry ether and the bulky precipitate which separated allowed to settle. The ethereal liquid was then syphoned off and the precipitate again dissolved in alcohol and the precipitation repeated. The ethereal solution was taken to dryness, the residue taken up in alcohol and again precipitated by dry ether. In this manner there were finally obtained two fractions: (a), soluble in alcohol and precipitated by ether, an amorphous light coloured, non-hygroscopic, water soluble powder; (b), a resinous product soluble in ether and in alcohol.

Of fraction (a) 4.9 grams were obtained of which the m. l. d. was found to be 0.15 mg. per 100 gram (frog) and which therefore represented about one-third of the original activity. It was observed that the aqueous solution of this substance (a) on warming became opaque owing to the separation of oily drops. The substance was therefore dissolved in about four to five parts of cold water and kept in a boiling water bath for about half an hour when the oily product settled to the bottom of the vessel and the clear supernatant liquid was syphoned off. On evaporation the solution yielded a deep yellow coloured solid which was not

hygroscopic, could be readily powdered and was found to be of a very high degree of toxicity. 0.03 mg. injected into a frog of 25 grams weight produced death within less than three hours, the substance thus possessing an m. l. d. of 0.12 mg. per 100 gram (frog). About 70 per cent of the original water soluble product was completely soluble in hot water.

The final product thus obtained was free from nitrogen, readily soluble in cold water, methyl, ethyl, or amyl alcohol, acetic acid and pyridine, but almost insoluble in ether, chloroform, ethyl acetate and the other usual organic solvents. The aqueous solution possesses an extremely bitter taste. With sulphuric acid the solid gives a brown colouration which on careful dilution gives first a purple violet colour and on further dilution passes through a rose red colour to a green solution from which finally a greyish green flocculent precipitate separates (cf. strophanthin reaction). The aqueous solution of the solid gives no precipitate with solutions of the heavy metals, e.g., mercuric chloride, lead acetate, etc., nor with tannic acid but the substance (again like the strophanthins may be salted out by saturation with ammonium sulphate. Alcoholic solutions treated with alcoholic lead acetate give no precipitate even on long standing. All attempts to crystallise this product have so far failed but experiments in this direction are being continued.

On hydrolysis with dilute acids the solution of this water soluble product becomes turbid very rapidly even at the ordinary temperature and a resinous product separates, while the solution acquires reducing properties which are due to the liberation of a sugar which under the usual treatment yields d-phenyl glucosazone.

The resinous product (b) soluble in ether containing a small proportion of alcohol, which represents approximately two-thirds of the original activity of the squill extract, appears to be a considerably purer form of Merck's scillitoxin. That this is so appears probable since scillitoxin by suitable treatment yields a product very similar in all respects to the resinous product (b). Thus if scillitoxin is dissolved in absolute alcohol (scillitoxin is by no means completely soluble in absolute alcohol) and the alcoholic

solution precipitated by pouring into 10 to 12 volumes of dry ether, the product remaining in solution in the ether-alcohol mixture possesses an m. l. d. which is approximately one-half that of the original scillitoxin. This product is thus of the same order of activity as the resinous product (b), has similar solubilities, viz., readily soluble in absolute alcohol, soluble in alcohol-ether mixture containing 7 to 10 per cent of alcohol, almost insoluble in water. Moreover both preparations on treatment with concentrated sulphuric acid and careful dilution with water give a very distinct green coloration (a reaction which differs from that given by the water soluble product in that there is no initial red or violet colour) similar to that given by digitoxine. It appears unlikely that the toxicity of scillitoxin can be due to the presence of any of the water soluble substance since the preparation is only soluble in water to a very slight extent and the characteristic colour reaction is completely absent.

It seems indeed most probable that the activity of squills is due to the presence of at least two active principles, one of which is very readily soluble in water, the other only very slightly.

## ISOLATION OF CAFFEINE FROM SQUILLS

No attempt has been made to carry out a complete chemical investigation of squills, but during the course of the work the interesting discovery was made of the existence of caffeine in the squill bulb.

The isolation of the base was accomplished as follows. The dry powdered squills (10 kilos) were extracted with hot 97 per cent alcohol and the extract concentrated to small bulk (1300 cc.). After filtering from precipitated resin the liquid was completely precipitated by basic lead acetate solution. The excess of lead was removed by means of sulphuric acid and the excess of sulphuric acid by baryta. The clear yellow filtrate was concentrated to a thin syrup (400 cc.) and thoroughly shaken out with amyl alcohol. The alcoholic solution was evaporated to dryness and the gummy residue dissolved in absolute alcohol and taken to dryness. After repeating this treatment a few times a crystalline

solid commenced to separate. The alcoholic solution was then allowed to stand for some time, and the solid then filtered off. After recrystallisation from alcohol, from which it separated in fine needles the substance was found to melt at 229° to 230°. It contained nitrogen was appreciably soluble in water, was not glucosidal but gave rather feeble alkaloidal reactions.

On analysis

0.1288 gave 0.0235 $CO_2$ and 0.0592 $H_2O$. C=49.8, H=5.1.
0.1152 gave 27.9 cc. $N_2$ (moist) at 12° and 770 mm. N=28.9
$C_8H_{10}O_2N_4$ requires C=49.5, H=5.1, N=28.8 per cent.

whence it was at once seen that the substance was in all probability caffeine. This was confirmed. The substance gave the murexide test, could be sublimed, and when mixed with a specimen of caffeine from another source the mixture melted at 231°.

The total amount of this base present in squills is however very small (approximately 0.01 per cent of the dry bulb) and from the pharmacological point of view is negligible in relation to the well known diuretic effect of the drug.

## SUMMARY

The toxicity of squills is in all probability due to the presence of at least two active principles.

1. A glucosidal substance very easily soluble in water, and resembling in many respects the water soluble strophanthin. This substance has been isolated in an approximately pure form but so far has not been crystallised. The minimum lethal dose of the product is 0.03 mg. for a frog of about 25 grams weight, death being produced by the stopping of the heart in systole.

2. A resinous product very slightly soluble in water, readily soluble in alcohol and not precipitated by ether from its alcoholic solution. The m. l. d. of this product is 0.06 to 0.07 mg. per frog.

From the alcoholic extract of the squill bulb a small quantity of caffeine was isolated. The base is present only in very small quantity (0.01 per cent of the dry bulb).